*Journal of Food Protection, Vol. 65, No. 6, 2002, Pages 999–1004*


# Concentration and Detection of Caliciviruses from Food Contact Surfaces

ANIL TAKU,[1] BALDEV R. GULATI,[2] PAUL B. ALLWOOD,[3] KERRIN PALAZZI,[4] CRAIG W. HEDBERG,[4] AND SAGAR M. GOYAL[1]*

[1]*Department of Veterinary Diagnostic Medicine, University of Minnesota, 1333 Gortner Avenue, St. Paul, Minnesota 55108, USA;* [2]*Department of Veterinary Microbiology, Haryana Agricultural University, Hisar, India;* [3]*Minnesota Department of Health, Metro Square Building, 121 East 7th Place, St. Paul, Minnesota 55164, USA; and* [4]*Department of Environmental and Occupational Health, University of Minnesota, 420 Delaware Street S.E., Minneapolis, Minnesota 55455, USA*

MS 01-384: Received 11 October 2001/Accepted 7 February 2002



## ABSTRACT

Outbreaks of human Norwalk virus (NV) and Norwalk-like viruses often originate in food service establishments. No reliable method is available for the detection of these human caliciviruses on food contact surfaces. We describe a simple method for the detection of NV from stainless steel work surfaces using cultivable feline calicivirus (FCV) as a model. Stainless steel surfaces were artificially contaminated with known amounts of FCV, followed by its elution in a buffer solution. Three methods of virus elution were compared. In the first method, moistened cotton swabs or pieces of nylon filter (1MDS) were used to elute the contaminating virus. The second method consisted of flooding the contaminated surface with eluting buffer, allowing it to stay in contact for 15 min, followed by aspiration of the buffer (aspiration method) after a contact period of 15 min. The third method, the scraping-aspiration method, was similar to the aspiration method, except that the surfaces were scraped with a cell scraper before buffer aspiration. Maximum virus recovery (32 to 71%) was obtained with the scraping-aspiration method using 0.05 M glycine buffer at pH 6.5. Two methods (organic flocculation and filter adsorption elution) were compared to reduce the volume of the eluate recovered from larger surfaces. The organic flocculation method gave an average overall recovery of 55% compared to the filter-adsorption-elution method, which yielded an average recovery of only 8%. The newly developed method was validated for the detection of NV by artificial contamination of 929-cm$^2$ stainless steel sheets with NV-positive stool samples and for the detection of the recovered virus by reverse transcription–polymerase chain reaction.

Norwalk (NV) and Norwalk-like viruses (NLVs) are a leading cause of foodborne gastroenteritis. Recently, it has been determined that 96% of the reported outbreaks of nonbacterial gastroenteritis in the United States are caused by NLVs *(11).* It is further estimated that NLVs account for more than 60% of the foodborne disease outbreaks due to known agents *(26).* The transmission of NLVs occurs mostly through the fecal-oral route, person-to-person transmission, or ingestion of contaminated foods. Transmission by aerosols has also been reported *(24).* The consumption of contaminated food is a common mode of NLV transmission *(4, 8),* and food handlers are often suspected to have a role in transmission, both in symptomatic and asymptomatic phases of the disease *(22, 28).* Various food items that have been implicated in NLV outbreaks include fresh fruits and vegetables, frozen raspberries, bakery products, sliced deli ham, deli sandwiches, shellfish, and salads *(7, 8, 11, 29).* In many produce-associated outbreaks, food and food contact surfaces were shown to be contaminated by infected food handlers while food items were being handled *(7, 8).*

Despite the possible involvement of different foods in the transmission of these viruses, efforts have so far focused on the detection of NLVs only in shellfish *(3, 9, 16, 21).* Recently, efforts toward developing methods for the detection of NLVs from other foods have also been initiated *(20, 31).* However, food contact surfaces have not been investigated for their role in the transmission of NLVs, possibly because effective methods are not available for virus recovery from such surfaces.

Several human enteric viruses, e.g., hepatitis A, human rotavirus, enteric adenovirus, and poliovirus, are known to survive on aluminum, china, glass, glazed tile, latex, and polystyrene surfaces *(1).* The transfer of viable viruses from contaminated to clean surfaces has also been documented *(2, 17).* Routine interaction of hands with contaminated surfaces has been shown to promote the spread of hepatitis A virus *(25).* These studies provide enough circumstantial evidence that, in restaurant-related outbreaks of NLVs, food contact surfaces may act as an important vehicle for virus transmission.

The objective of this study was to develop a simple method for the detection of feline calicivirus (FCV) from food contact surfaces. Because NLVs do not grow in cell cultures, we used cultivable FCV as a surrogate model. NV is a calicivirus, and its physicochemical properties are similar to those of FCV *(32).* It is therefore appropriate to use FCV as a surrogate for NLV. In a similar fashion, poliovirus has been successfully used as a surrogate model for developing methods for the detection of human enteric viruses

* Author for correspondence. Tel: 612-625-2714; Fax: 612-624-8707; E-mail: goyal001@umn.edu.

from water, shellfish, and sludge *(14)*. Recently, primate calicivirus (Pan-1) has also been used successfully as a surrogate for developing detection methods for human caliciviruses in water *(18)*.

## MATERIALS AND METHODS

**Experimental plan.** Demarcated areas of stainless steel surfaces were contaminated with known amounts of FCV. To elute the contaminating virus from surfaces, three different buffer solutions and three different elution methods were compared to determine the efficiency of virus recovery. Initially, the methods were tested on small surfaces (25.8 and 929 $cm^2$), followed by the use of larger surfaces (bench tops and sinks). Once the contaminated virus was eluted from larger surfaces (5,290 $cm^2$), the volume of the eluate was further reduced by one of two virus concentration methods: (i) the membrane adsorption-elution method using 1MDS positively charged filters (CUNO, Meriden, Conn.) *(15)*, or (ii) the organic flocculation method *(19)*. The newly developed method was then validated for the detection of FCV and NV by reverse transcription–polymerase chain reaction (RT-PCR) by contaminating surfaces with serial 10-fold dilutions of FCV or NV, recovering the applied virus by the newly developed method, and detecting them by RT-PCR.

**Viruses.** Strain F9 of FCV was a gift of John Neill, National Animal Disease Center, Ames, Iowa. A fecal sample containing NV was obtained from Joanne Bartkus, Department of Health, Minneapolis, Minn. The NV from the fecal sample clustered with genogroup I isolates, specifically with the P1-A antigenic group. A 10% (wt/vol) suspension of fecal sample in phosphate-buffered saline was vortexed and centrifuged at 16,000 × *g* for 5 min. The supernatant was aliquoted and stored at −70°C. A fecal sample that did not show the presence of NV by RT-PCR was reconstituted and processed in a similar manner and was used as a negative control.

**Cells.** The FCV was propagated and titrated in Crandell-Reese feline kidney (ATCC CCL94) cells (American Type Culture Collection, Rockville, Md.). The cells were grown in Eagle's minimal essential medium (Celox, St. Paul, Minn.) supplemented with 8% fetal bovine serum, penicillin (100 U/ml), streptomycin (100 μg/ml), fungizone (1 μg/ml), 15 mM *N*-2-hydroxyethylpiperazine*N*′-2-ethanesulfonic acid (HEPES), and 5 mg/ml lactalbumin hydrolysate. Monolayers at 90% confluency were inoculated with FCV, and the cells were incubated at 37°C until cytopathic effects were seen, usually within 2 to 3 days. The cultures were frozen and thawed three times, followed by centrifugation at 12,000 × *g* for 5 min. The supernatant was aliquoted in 1-ml amounts and stored at −70°C. For virus titration, 100 μl of serial 10-fold dilutions of the samples was inoculated in triplicate in Crandell-Reese feline kidney monolayers in 96-well microtiter plates. After 72 h of incubation, the cells were observed for cytopathic effects, and the 50% tissue culture infective dose ($TCID_{50}$) was calculated by the method of Reed and Muench *(30)*.

**Experimental contamination of surfaces.** Stainless steel sheets (Type 304 ss, no. 4 polished stainless steel) of 929-$cm^2$ area were fabricated locally (Crystal Welding, Maple Grove, Minn.). Demarcated areas on these sheets were used as representatives of the environmental food contact surfaces that are likely to come in contact with food or food handlers in food service establishments. Stainless steel tabletops, sinks, and metal push plates on doors were also used in certain experiments. These surfaces were contaminated with known amounts of FCV, which was then allowed to dry for 15 min at room temperature. To reduce operator bias, the person collecting the surface samples did not know the exact location of virus application on these surfaces.

**Virus elution.** Initially, three buffers were used (0.05 M glycine buffer, pH 6.5; 0.05 M glycine buffer, pH 9.5; and modified Eagle's medium, pH 7.2). Three different methods of virus elution using only one buffer were compared. The first method consisted of swabbing the contaminated surface with moistened swabs (Cotton Tails, Citmed, Mobile, Ala.) or pieces of 1MDS filter wrapped around the tips of a forceps. The swabs or filters moistened with 0.05 M glycine buffer (pH 6.5) were firmly rubbed 20 to 25 times across the length and breadth of the contaminated surface. The swabs and filter pieces were vortexed for 30 s in 1 ml of 0.05 M glycine buffer (pH 9.5) to elute the recovered virus. The aspiration method consisted of pouring the eluting buffer evenly over the surface (10 ml buffer/929-$cm^2$ area), followed by vacuum aspiration of the buffer after a contact time of 15 min. The scraping-aspiration method was the same as the aspiration method, except that the surfaces were firmly mopped with a sterile cell scraper (with a 3-cm blade; Costar, Corning, N.Y.) before aspiration of the eluting buffer. For vertical surfaces, the buffer was evenly sprayed with a hand-held sprayer bottle and then collected in a sterile reservoir with the cell scraper. For sinks, the sinkhole was blocked with a rubber stopper before applying the virus. The eluting buffer was then sprayed on the walls and the bottom of the sink, and the eluate was allowed to collect around the blocked outlet, where it was aspirated into sterile flasks. All eluates were adjusted immediately to pH 7.2 to 7.4 and assayed for FCV with (for 100-ml eluates) or without (10-ml eluates) concentration.

**Virus concentration.** Two methods were compared for reducing the eluate volume. For the organic flocculation method, beef extract powder (Becton Dickinson, Cockeysville, Md.) was added to the eluates at a final concentration of 3%, and pH was adjusted to 3.5 with 1 N HCl. The mixture was stirred for 20 min to allow flocculation and then centrifuged at 4,000 × *g* for 20 min. The pellet was suspended in 1 ml phosphate-buffered saline. The filter-adsorption-elution method was performed in two steps. First, the eluate was passed through a double layer of 25-mm-diameter 1MDS filter at a flow rate of 1- to 2-ml/min/$cm^2$ filter area. In the next step, virus bound to the filter was eluted with 1 ml of 0.05 glycine buffer containing 3% beef extract (pH 9.5). The filter was allowed to soak in the eluent for 1 to 2 min before the eluent was passed through the filter. The pH of the filter eluate was adjusted to 7.2 to 7.4, and the eluate was titrated for FCV or tested by RT-PCR.

**Decontamination.** At the end of an experiment, the stainless steel sheets were autoclaved before reuse. Other contaminated surfaces (bench tops, sinks, and push plates on doors) were decontaminated with a 10% solution of bleach (Fox-chlor, Fox Packaging Co., St. Paul, Minn.), which was allowed to stay in contact with the surfaces for 2 to 4 h. The surfaces were then washed with water and dried.

**RNA extraction.** The QIAamp viral RNA mini kit (Qiagen, Valencia, Calif.) was used for extraction and purification of viral RNA per manufacturer's instructions. Briefly, 140 μl of concentrated sample was mixed with 560 μl buffer and incubated at room temperature for 10 min for lysis. Ethanol (560 μl) was then added and mixed by vortexing, and droplets from the centrifuge tube cap were removed by centrifugation. The QIAamp spin column was then loaded with 630 μl of this mixture and centrifuged. The filtrate was discarded, and the spin column was washed with 500 μl buffer, followed by another wash with 500 μl buffer. The filtrate was discarded, and the RNA bound to the spin column was

TABLE 1. *Elution of FCV from stainless steel surfaces using different eluents*[a]

| | % virus recovered | | | |
|---|---|---|---|---|
| | Trial 1 | Trial 2 | Trial 3 | Average |
| 0.05 M glycine (pH 6.5) | 50 | 36 | 40 | 42 |
| 0.05 M glycine (pH 9.5) | 35 | 20 | 29 | 28 |
| Modified Eagle's medium (pH 7.2) | 10 | 5 | 14 | 10 |

[a] Twenty microliters of FCV (1.9 to 3.6 × $10^6$ $TCID_{50}$/100 μl) was spotted on 929-$cm^2$ stainless steel sheets and allowed to dry for 15 min. The contaminated sheets were flooded with 10 ml of the indicated buffer, allowed to stay in contact for 15 min, scraped with a cell scraper, aspirated by vacuum aspiration, and then titrated for FCV. Percent virus recovery was calculated by the following formula: (Amount of virus recovered in the eluate)/(Amount of virus used for contamination) × 100.

eluted with 30 μl RNase- and DNase-free distilled water (Life Technologies, Rockville, Md.). RNA was either used directly in RT-PCR or stored at −70°C.

**RT-PCR.** FCV RNA was detected by RT-PCR using primer pairs CalcapF and CalcapR *(34),* which generate a 673-bp product. NV RNA was detected by primer pair JV12 and JV13, which generates a 326-bp product *(36).* Briefly, 5 μl RNA was reverse transcribed in a total volume of 20 μl. The RT mixture contained 20 mM Tris-HCl, 50 mM KCl, 6.25 mM $MgCl_2$, 2 μM downstream primer, 1 mM each of deoxynucleoside triphosphates, 5 mM dithiothreitol, 20 U RNase inhibitor (Life Technologies), and 20 U Superscript II (Life Technologies). RT was performed at 42°C for 45 min, followed by inactivation at 70°C for 15 min. PCR amplification of 5 μl cDNA was performed in a 50-μl reaction volume containing 20 mM Tris-HCl, 50 mM KCl, 2 mM $MgCl_2$, 200 μM deoxynucleoside triphosphates, 0.4 μM of each primer, and 2.5 U *Taq* DNA polymerase (Life Technologies). Amplification was performed in a Gene Amp PCR system 9600 (Perkin Elmer, Norwalk, Conn.) with an initial denaturation at 94°C for 2 min, followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min, and a final extension at 72°C for 10 min. The RT-PCR products were analyzed by agarose gel electrophoresis, stained with ethidium bromide, and visualized under UV light.

## RESULTS

**Comparison of elution buffers.** To find an optimum eluent, we compared 0.05 M glycine buffer at pH 6.5 and 9.5. Modified Eagle's medium at pH 7.2 was also used. Using a 929-$cm^2$ surface area, the average virus recoveries with the three buffers were 42, 28, and 10%, respectively (Table 1). Because consistently higher recoveries of FCV were obtained with 0.05 M glycine at pH 6.5, this buffer was used in all subsequent experiments.

**Method of virus elution.** Four different methods of virus elution were then compared for eluting contaminating FCV from 25.8- and 929-$cm^2$ surfaces. As shown in Table 2, an average virus recovery of less than 23% was obtained with cotton or 1MDS filter swabs. Similar virus recoveries were obtained when the eluting buffer was aspirated without scraping. However, as much as 71% of the input virus was recovered when virus release was facilitated by scraping the surface with cell scrapers before buffer aspiration. The scraping-aspiration method was then applied to recover FCV from sinks and push plates on doors. Average recoveries of 34 and 11%, respectively, were obtained.

**Concentration of eluates.** Next, we contaminated stainless steel laboratory bench tops (929-$cm^2$ area) and eluted the contaminating virus by the scraping-aspiration method using 100 ml of 0.05 M glycine buffer (pH 6.5).

TABLE 2. *Comparison of elution methods for the recovery of FCV from surfaces*

| Surface | Average % (range) virus recovered by the indicated elution method[a–c] | | | |
|---|---|---|---|---|
| | Cotton swabs | 1MDS swabs | Aspiration | S/A |
| Stainless steel sheet (25.8 $cm^2$)[d] | 10 (6–16) | 23 (11–30) | 25 (15–35) | 71 (58–90) |
| Stainless steel sheet (929 $cm^2$)[d] | 3 (2.5–4.5) | 2.5 (1.0–5.0) | 8.6 (6–12) | 32 (20–52) |
| Sink[e] | ND | ND | ND | 34 (20–46) |
| Vertical surface[f] | ND | ND | ND | 11 (8–15) |

[a] Percent virus recovery was calculated by the following formula: (Amount of virus recovered in the eluate)/(Amount of virus used for contamination) × 100.

[b] The numbers in parentheses indicate range of virus recovery in three different experiments.

[c] S/A, scraping-aspiration (procedure); ND, not done.

[d] Twenty microliters of FCV (7.0 × $10^5$ to 1.3 × $10^6$ $TCID_{50}$/100 μl) was spotted on the indicated surfaces at random spots and allowed to dry for 15 min. For the first two procedures, the contaminated surface was swabbed with moistened cotton swabs or 1MDS filter swabs, followed by rinsing in 1 ml of 0.05 M glycine buffer (pH 6.5) to recover the eluted virus. For the aspiration procedure, the contaminated surface was flooded with 0.05 M glycine buffer (pH 6.5) at 10 ml/929 $cm^2$, allowed to stay in contact for 15 min, and then aspirated. The S/A procedure was similar to the aspiration procedure, except that the contaminated surface was scraped with a cell scraper before vacuum aspiration of the eluent.

[e] The sinkhole was blocked. The inner surfaces were spotted with 20 μl FCV and allowed to dry. The walls were then uniformly sprayed with 0.05 M glycine buffer (pH 6.5). After a contact period of 15 min, the walls and base of the sink were scraped; the eluent was then allowed to collect near the blocked sinkhole and was aspirated by vacuum aspiration.

[f] Metal push plates on doors were spotted with 20 μl FCV and allowed to dry. The push plate was then sprayed uniformly with 5 ml of 0.05 M glycine buffer (pH 6.5). The buffer was scraped from the surface using cell scrapers and collected in a reagent reservoir held underneath.

TABLE 3. *Comparison of organic flocculation and filter-adsorption-elution methods for second-step concentration of FCV eluted from contaminated surfaces*[a]

| Method | % virus recovered | | | |
|---|---|---|---|---|
| | Trial 1 | Trial 2 | Trial 3 | Average |
| Organic flocculation[b] | 70 | 48 | 47 | 55 |
| Filter adsorption elution[c] | 12 | 5 | 8 | 8 |

[a] Ten microliters of FCV (1.0 to 7.5 × $10^5$ $TCID_{50}$/100 μl) was applied at random spots on a 929-$cm^2$ stainless steel bench top and allowed to dry. The contaminated surface was flooded with 100 ml of 0.05 M glycine buffer (pH 6.5). The surface was then scraped with sterile cell scrapers, and the buffer was collected by vacuum aspiration. Virus present in the eluate was concentrated either by organic flocculation or by filter adsorption elution.

[b] Organic flocculation was done by adding beef extract powder to the eluate at a final concentration of 3% and adjusting the pH to 3.5. The mixture was stirred for 20 min to allow flocculation and then centrifuged at 4,000 × *g* for 20 min. The pellet was suspended in 1 ml phosphate-buffered saline (pH 7.2) and titrated for FCV.

[c] The filter-adsorption-elution method was performed by passing the eluate through 1MDS filter, followed by virus elution in 1 ml of 0.05 M glycine buffer containing 3% beef extract (pH 9.5). The samples were adjusted to pH 7.2 to 7.4 before inoculation in cell cultures for FCV titration.

To reduce the volume of the eluate to a manageable level, we compared two methods (organic flocculation and filter adsorption elution) for the concentration of the eluted virus. The average virus recovery with these two methods was 55 and 8%, respectively (Table 3).

**Detection of viruses by RT-PCR.** Finally, we validated the newly developed method for the detection of FCV and NV by RT-PCR. Using the scraping-aspiration method followed by organic flocculation for concentration, we were able to detect the presence of FCV in up to $10^{-5}$ dilution of the stock FCV with a detection limit of 2.1 × $10^1$ $TCID_{50}$ (Table 4). This was also the last dilution that gave a positive reaction by RT-PCR. Similarly, 100 μl of 10-fold dilutions of NV-positive stool samples was used to contaminate 929-$cm^2$ stainless steel sheets. The NV dilutions were collected by the scraping-aspiration method, concentrated to 1 ml by organic flocculation, and subjected to RT-PCR. With conserved capsid primers JV12/JV13 *(36),* we could detect up to $10^{-5}$ dilution of NV-positive stool samples collected from artificially contaminated stainless steel plates.

## DISCUSSION

Outbreaks of gastroenteritis caused by NLVs have become a major public health concern. Effective control of outbreaks depends in part on the identification of the source of contamination. The contamination of raw produce by contact with infected food handlers or contaminated food contact surfaces has serious implications for the food service industry and for large eating establishments. Thus, simple methods for the detection of human caliciviruses from food contact surfaces are needed. We have described the development of a simple method for the detection of NV in artificially contaminated food contact surfaces using FCV as a model virus.

Because the pH and ionic strength of eluting buffer are known to influence the net charge on virus particles *(14, 15),* it is important to determine which eluting buffer is the most efficient. In general, eluents of low ionic strength are suitable for the elution of enteric viruses. We found 0.05 M glycine buffer (pH 6.5) to be the most suitable for the elution of FCV. The same buffer at pH 9.5 was less efficient for virus recovery, and modified Eagle's medium was the least efficient, probably because of its complex composition.

Initially, mopping of the surface with a swab or pieces of 1MDS filter was evaluated as a sampling method because of its ease of operation. However, this method yielded consistently poor recovery of the input virus, probably because the size of the swab head was inadequate to retain the buffer and, thus, the eluted virus. It is also likely that the spotted virus, after coming in contact with the swab, gets spread and smeared along the entire surface, resulting in virus dilution per unit surface area. Studies with hepatitis B virus antigens on environmental surfaces have shown the swab rinse method to be reliable, but the efficacy of this method is reported to be the greatest when the area to be sampled is small and when the level of contamination is high *(5, 10, 12).* In a study on microbial contamination of spacecraft hardware, it was recommended that the area sampled with a cotton swab should not exceed 25.4 $cm^2$ *(27).*

TABLE 4. *Efficiency of detection of feline calicivirus (FCV) and Norwalk virus (NV) by reverse transcription–polymerase chain reaction (RT-PCR)* [a]

| Dilution of virus used to contaminate 929-$cm^2$ area | $TCID_{50}$ of FCV in the indicated dilution | Detection of indicated dilution of FCV by RT-PCR | Detection of indicated dilution of NV by RT-PCR |
|---|---|---|---|
| $10^{-1}$ | 2.1 × $10^5$ | + | + |
| $10^{-2}$ | 3.1 × $10^4$ | + | + |
| $10^{-3}$ | 2.1 × $10^3$ | + | + |
| $10^{-4}$ | 4.5 × $10^2$ | + | + |
| $10^{-5}$ | 2.1 × $10^1$ | + | + |
| $10^{-6}$ | — | − | − |

[a] Serial 10-fold dilutions of FCV (initial titer = 2.1 × $10^6$ $TCID_{50}$/20 μl) and NV were spotted on 929-$cm^2$ stainless steel bench tops. The virus was eluted by the scraping-aspiration method and concentrated down to 1 ml by organic flocculation. The final samples were titrated by cell cultures and RT-PR (for FCV) and by RT-PCR (for NV).

Our goal was to sample large surfaces of restaurant kitchens for which commercially available swabs and sample collection proved ineffective. Sampling large areas of food contact surfaces with nonbactericidal cellulose sponges impregnated in nutrient broth has been recommended *(13),* and it seems that sponging may be a versatile tool for sampling since it can cover both even and uneven surfaces (e.g., food service equipment). Sponging as a sampling tool was not used in the present study but should be evaluated as a tool for sampling large environmental surfaces for caliciviruses.

We then attempted to improve virus recovery by aspirating the eluting buffer after a contact time of 15 min. This method also did not give satisfactory yields of contaminating FCV. It has been demonstrated earlier that the transfer of viruses between contaminated and clean surfaces is high when the inoculum is wet *(2, 17).* Thus, rinsing and aspiration of eluting buffer from the surface to be sampled may have the advantage of avoiding desiccation of the virus, but virus recovery with this method was poor.

The aspiration method was modified by allowing the elution buffer to stay in contact with the contaminated surface for 15 min. The surface was then mopped with a cell scraper to facilitate the release of virus into the eluting buffer. This method gave an average virus recovery of 32 to 71% with horizontal surfaces and the sink. That the scraping-aspiration method proved to be the best of all methods is not surprising, because stainless steel is moderately hydrophilic with negative surface charge, and irreversible attachment of microorganisms to stainless steel may occur within 1 min *(23, 35),* making it potentially difficult to elute viruses once they are absorbed on such surfaces. In a study on the survival of parainfluenza virus on fomites, it was reported that vigorous cleaning was as important as disinfection in removing contaminating viruses from stainless steel surfaces *(6).* In addition, stainless steel surfaces may be marked with scratches or crevices due to wear and tear, as well as to polishing with abrasive powders or substances. These crevices or channels may serve to entrap food residues and microorganisms *(33).* It is probably for this reason that scraping the virus-contaminated surface and aspirating the buffer gave better recovery, probably by physically dislodging the viral particles.

The average recovery with vertical surfaces was only 11%, probably because the buffer was not retained long enough on these surfaces for the scraped virus to become solubilized. Sampling from the sinks, by contrast, yielded 34% of the input virus, because the scraped buffer from the vertical walls of the sinks could be retained by blocking the sinkhole and could be reused to allow optimum contact time with the contaminated surfaces.

The sampling of large surface areas is important to maximize virus recovery. This can be achieved by larger amounts of eluting buffer, so that the whole surface comes in contact with the eluting buffer. The large amounts of eluate thus obtained (ca. 100 ml or more) must be concentrated down to a small amount that can be easily assayed in cell cultures or by RT-PCR. For this purpose, the organic flocculation method was found to be appropriate. The filter-adsorption-elution method was found to be impractical, because the filters became easily clogged by solid matter picked up during elution. Organic flocculation as a method of concentration has the advantage of precipitating the virus from particulate matter that might clog the filters. Another advantage of using organic flocculation is that the sedimented pellet is compact and can be reconstituted in a small volume of buffer. The organic flocculation method has been extensively used as a second-step concentration method for the concentration and detection of viruses from water *(14, 15, 18).*

The detection limit for FCV was found to be $2.1 \times 10^1$ $TCID_{50}$ by both cell culture and RT-PCR. Similarly, RT-PCR of NV dilutions collected from artificially contaminated steel surfaces showed a positive RT-PCR reaction for up to $10^{-5}$ dilution of an NV-positive stool sample. The initial concentration of NV in this sample was not known; hence, we cannot comment on the sensitivity of RT-PCR for NV detection. However, it appears that the newly developed method can also detect NV from environmental surfaces. The method is simple to perform and does not require any special equipment. Studies are in progress to further refine this method and adapt it to examine other food contact surfaces, e.g., interiors of refrigerators, telephones, levers, switches, door handles, doorknobs, cutlery, and other equipment.

## ACKNOWLEDGMENTS

This study was supported in part by the Cooperative State Research, Education, and Extension Service, U.S. Department of Agriculture, under agreement no. 99-04862. We thank Sigrun Haugerud, Wendy Wiese, and Amanda Foss for technical assistance.

## REFERENCES

1. Abad, F. X., R. M. Pinto, and A. Bosch. 1994. Survival of enteric viruses on environmental fomites. Appl. Environ. Microbiol. 60: 3704–3710.
2. Ansari, S. A., S. A. Sattar, V. S. Springthrope, G. A. Wells, and W. Tostowaryk. 1988. Rotavirus survival on human hands and transfer of infectious virus to animate and non-porous inanimate surfaces. J. Clin. Microbiol. 26:1513–1518.
3. Atmar, R. L., F. H. Neill, J. L. Romalde, F. Le Guyader, C. M. Woodley, T. G. Metcalf, and M. K. Estes. 1995. Detection of Norwalk virus and hepatitis A virus in shellfish tissues with the PCR. Appl. Environ. Microbiol. 61:3014–3018.
4. Blacklow, N. R., J. E. Herrmann, and W. D. Cubitt. 1987. Immunobiology of Norwalk virus. Ciba Found. Symp. 128:144–161.
5. Bond, W. W., N. J. Petersen, and M. S. Favero. 1977. Viral hepatitis B: aspects of environmental control. Health Lab. Sci. 14:235–252.
6. Brady, M. T., J. Evans, and J. Cuartas. 1990. Survival and disinfection of parainfluenza viruses on environmental surfaces. Am. J. Infect. Control 18:18–23.
7. Daniels, N. A., D. A. Bergmire-Sweat, K. J. Schwab, K. A. Hendricks, S. Reddy, S. M. Rowe, R. L. Fankhauser, S. S. Monroe, R. L. Atmar, R. I. Glass, and P. Mead. 2000. A foodborne outbreak of gastroenteritis associated with Norwalk-like viruses: first molecular traceback to deli sandwiches contaminated during preparation. J. Infect. Dis. 181:1467–1470.
8. Deneen, V. C., J. M. Hunt, C. R. Paule, R. I. James, R. G. Johnson, M. J. Raymond, and C. W. Hedberg. 2000. The impact of foodborne calicivirus disease: the Minnesota experience. J. Infect. Dis. 181(Suppl. 2):S281–283.
9. Dix, A. B., and L. A. Jaykus. 1998. Virion concentration method for

the detection of human enteric viruses in extracts of hard-shelled clams. J. Food Prot. 61:458–465.
10. England, B. L. 1982. Detection of viruses on fomites, p. 179–220. *In* C. P. Gerba and S. M. Goyal (ed.), Methods in environmental virology. Marcel Dekker, New York.
11. Fankhauser, R. L., J. S. Noel, S. S. Monroe, T. Ando, and R. I. Glass. 1998. Molecular epidemiology of "Norwalk-like viruses" in outbreaks of gastroenteritis in the United States. J. Infect. Dis. 178: 1571–1578.
12. Favero, M. S., W. W. Bond, N. J. Petersen, and K. R. Berquist. 1974. Detection methods for the study of the stability of hepatitis B antigen on surfaces. J. Infect. Dis. 129:210–212.
13. Food and Safety Inspection Service, 1998, "Guidelines for Environmental and Chill Water/Brine Sampling for *Listeria,*" [Internet, WWW], ADDRESS: http://fsis.usda.gov/S/rolab/envsamp.htm.
14. Gerba, C. P., and S. M. Goyal (ed.). 1982. Methods in environmental virology. Marcel Dekker, New York.
15. Goyal, S. M., and C. P. Gerba. 1983. Viradel method for detection of rotavirus from seawater. J. Virol. Methods 7:279–285.
16. Hafliger, D., P. Hubner, and J. Luthy. 2000. Outbreak of viral gastroenteritis due to sewage-contaminated drinking water. Int. J. Food Microbiol. 10:123–126.
17. Hall, C. B., G. Douglas, Jr., and J. M. Geiman. 1980. Possible transmission by fomites of respiratory syncytial virus. J. Infect. Dis. 141: 98–102.
18. Huang, P. W., D. Laborde, V. R. Land, D. O. Maston, A. W. Smith, and X. Jiang. 2000. Concentration and detection of caliciviruses in water samples by reverse transcription PCR. Appl. Environ. Microbiol. 66:4383–4388.
19. Katzenelson, E., B. Fattal, and T. Hostovesky. 1976. Organic flocculation: an efficient second-step concentration method for the detection of viruses in tap water. Appl. Environ. Microbiol. 32:638–639.
20. Leggitt, P. R., and L. A. Jaykus. 2000. Detection methods for human enteric viruses in representative foods. J. Food Prot. 63:1738–1744.
21. Le Guyader, F., M. K. Estes, M. E. Hardy, F. H. Neill, J. Green, D. W. Brown, and R. L. Atmar. 1996. Evaluation of a degenerate primer for the PCR detection of human caliciviruses. Arch. Virol. 141: 2225–2235.
22. Lo, S. V., A. M. Conolly, S. R. Palmer, D. Wright, P. D. Thomas, and D. Joynson. 1994. The role of pre-symptomatic food handler in a common source outbreak of food-borne SRSV gastroenteritis in a group of hospitals. Epidemiol. Infect. 113:513–521.
23. Mafu, A. A., D. Roy, J. Goulet, and L. Savoie. 1991. Characterization of physicochemical forces involved in adhesion of *Listeria monocytogenes* to surfaces. Appl. Environ. Microbiol. 57:1969–1973.
24. Marks, P. J., I. B. Vipond, D. Carlisle, D. Deakin, R. E. Fey, and E. O. Caul. 2000. Evidence for airborne transmission of Norwalk-like virus (NLV) in a hotel restaurant. Epidemiol. Infect. 124:481–487.
25. Mbithi, J. N., V. S. Springthrope, J. R. Boulet, and S. A. Sattar. 1992. Survival of hepatitis A virus on human hands and its transfer on contact with animate and inanimate surfaces. J. Clin. Microbiol. 30: 757–763.
26. Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe. 1999. Food-related illness and death in the United States. Emerg. Infect. Dis. 5:607–625.
27. National Aeronautics and Space Administration. 1968. Standard procedures for microbiological examination of space hardware. NHB 5340. 1A. National Aeronautics and Space Administration, Washington, D.C.
28. Patterson, T., P. Hutchings, and S. Palmer. 1993. Outbreak of SRSV gastroenteritis at an international conference traced to food handled by a post symptomatic caterer. Epidemiol. Infect. 111:157–162.
29. Ponka, A., L. Maunula, C. H. von Bonsdorff, and O. Lyytikainen. 1999. An outbreak of calicivirus associated with consumption of frozen raspberries. Epidemiol. Infect. 123:469–474.
30. Reed, L. J., and H. Muench. 1938. Simple method for estimating fifty percent endpoints. Am. J. Hyg. 27:493–497.
31. Schwab, K. J., F. H. Neill, R. L. Fankhauser, N. A. Daniels, S. S. Monroe, D. A. Bergmire-Sweat, M. K. Estes, and R. L. Atmar. 2000. Development of methods to detect "Norwalk-like" viruses (NLVs) and hepatitis A virus in delicatessen foods: application to a food-borne NLV outbreak. Appl. Environ. Microbiol. 66:213–218.
32. Slomka, M. J., and H. Appleton. 1998. Feline calicivirus as a model system for heat inactivation studies of small round structured viruses in shellfish. Epidemiol. Infect. 121:401–407.
33. Speers, J. G. S., A. Gilmour, T. W. Fraser, and R. D. McCall. 1984. Scanning electron microscopy of dairy equipment surfaces. J. Appl. Bacteriol. 57:139–145.
34. Sykes, J. E., V. P. Studdert, and G. F. Browning. 1998. Detection and strain differentiation of feline calicivirus in conjunctival swabs by RT-PCR of the hypervariable region of capsid gene. Arch. Virol. 143:1321–1334.
35. Vanhaecke, E., J. P. Remon, M. Moors, E. Raes, F. De Rudder, and A. Van Peteghem. 1990. Kinetics of *Pseudomonas aeruginosa* adhesion to 304 and 316-I stainless steel, role of cell surface hydrophobicity. Appl. Environ. Microbiol. 56:788–795.
36. Vinje, J., and M. P. Koopmans. 1996. Molecular detection and epidemiology of small round-structured viruses in outbreaks of gastroenteritis in the Netherlands. J. Infect. Dis. 174:610–615.